PENICILLIUM SPICULISPORUM, A NEW ASCOGENOUS FUNGUS

S. G. Lehman

(With Plate 19)

The organism herein described first appeared in cultures from rootlets of apparently healthy cotton plants taken from a field in Anson County, N. C. In making the original cultures short pieces of rootlets were first washed, then treated with 50 per cent. solution of alcohol, rinsed in sterile water and placed into tubes of steamed rice. Some three or four weeks later the original cultures were observed to contain a fungus which had formed great numbers of perithecia of the type belonging to the Aspergillaceae. When ascospores from these perithecia were planted in potato glucose agar in petri dishes, white spreading colonies developed producing, first, a sparse crop of penicillate conidial fructifications and later, perithecia identical with those in the original cultures. The structure of the condial fructifications places this fungus definitely in the genus *Penicillium,* and a comparison of its morphological and cultural characters with those of other species of this genus as given in descriptions published by Saccardo (1), Lafar (2), Engler and Prantl (3), Thom (4), Sopp (5), Sartory (6) and Sartory and Bainier (7–10) shows it to differ from any of these in one or more important features. It is, therefore, described as a new species and since the walls of the ascopores bear minute spines, it is designated **Penicillium spiculisporum.**

Work with this fungus has shown that perithecia are produced in abundance on a wide variety of media without special cultural methods, a character rendering improbable the assumption that anyone could have cultivated this fungus without having observed them. This habit of abundant and continued perithecial formation coupled with sparse conidial production, a character not at all usual to species of *Penicillium,* is possessed in common

by *P. luteum* Zukal (11), *P. avellaneum* Thom and Turreson (12) and the species herein described. Its morphology, the production of transient yellow color in limited areas on certain media, and the general production of yellow associated with perithecia on potato plugs seem to place *P. spiculisporum* in the luteum-purpurogenum series as defined by Thom (1).

DESCRIPTION OF THE ORGANISM

Conidia sown on potato or bean agar and kept at 28° C. germinate in great numbers within ten hours by first swelling, then putting out one to three tubes (Fig. 18). These hyphae rapidly branch, and, by the end of 48 hours, form a growth easily visible to the eye. From these centers low spreading colonies develop with white floccose surface consisting of aerial mycelium bearing a sparse crop of conidiophores as short lateral branches. The mycelium is hyaline, septate, branched, often forked at the apex, 2–3.5 μ in diameter (Fig. 1–3). Numerous perithecial initials may appear within two weeks, giving the surface a granular appearance. White is the predominating surface color, but this may change to cream, yellow or pinkish as the perithecia mature. The yellow color is often transient, fading again to white or cream. Infrequently in tube cultures, portions of the surface are made to appear gray by a profuse development of conidia.

Conidiophores are short, 10–50 μ by 2–2.5 μ, usually 14–20 μ long, and bear a single verticel of three to five conidiiferous cells (Figs. 9–12). Occasionally there are only one or two or as many as six conidiiferous cells in a verticel (Fig. 5–8). Frequently the conidiophore produces a side branch bearing a single chain of conidia (Fig. 13) or, less frequently, it may carry at its summit two metulae each of which bear verticels of three to five conidiiferous cells (Fig. 14). If the conidial fructification occupies the end of an aërial hypha, the conidiiferous cells may be loosely disposed over a distance of 40–50 μ back from the apex (Fig. 15).

Conidiiferous cells are 11–16 μ long by 1.8–2.5 μ in diameter at the thickest part, the distal third of each cell tapering to a

sterigma of half this diameter (Fig. 7–9). Conidial chains may attain a length of 85 μ, easily break apart in water mounts and do not form columns. Conidia are ovate, elliptical or globose, 2.5–4 μ by 1.8–2.5 μ in six-day old drop cultures, have smooth walls and appear hyaline under the microscope (Fig. 16, 17). However, when they form in profusion, as infrequently happens in small areas, the surface appears gray with a faintly perceptible shade of green or brown according to the density of the growth. They swell and produce one to three tubes in germination.

If cultures on steamed rice and potato plugs in tubes, or on potato- and bean-glucose agar in petri dishes, are kept at 35 degrees Centigrade, great numbers of perithecia will be found developing at the end of two weeks. If crushed mounts are made at the end of 20 days, great numbers of asci and ascospores may be found in all stages of development. Perithecia are at first white, and may remain so; but, within thirty days, the color may have changed so that white, cream, pink and yellow shades may appear, all of which may be present in the same culture. The yellow is often transitory, fading again to cream or white.

Perithecial formation begins with the twisting together of the ends of two or more hyphae to form a knot (Fig. 19). Other hyphae grow out from and weave around this knot, gradually enlarging it and forming a white hyphal gnarl. When this gnarl has nearly attained mature size, the cells of the hyphal branches within swell and are transformed to asci (Fig. 23–28). When this process of development is complete, the perithecial cavity is found to be closely packed with ascospores, the delicate ascus walls and all hyphae having entirely disappeared. Perithecia are spherical, except when crowded, 0.4–2 mm. (mostly 0–5.1 mm.), indehiscent, with a peridium consisting of three parts: a thin inner layer of closely woven hyphae, a thick middle layer of loosely woven threads, and a very narrow outer layer (Fig. 20–22). The hyphae constituting the middle layer and the outermost layer of the peridium are of less diameter than those composing the innermost layer and the ascogenous center.

Asci are globose, elliptical, or pyriform, 7.2–10.8 by 6.3–7.7 μ, hyaline, 6–8-spored, the walls disappearing as the spores mature

(Fig. 30–34). Ascospores are ovate to elliptical, 2.5–4 μ by 1.8–2.8 μ, with walls bearing minute spines (Fig. 35). The spines are visible only under oil immersion, and then to best advantage when the spores have been treated three to five minutes with alcohol carbol fuchsin as if staining bacteria. Only about one per cent. of the ascospores have germinated on any of the various media used. Fig. 37 represents a colony grown from a single ascospore on beef peptone agar kept at 28° C. for 48 hours. At 72 hours, this colony had attained a diameter of 675 μ and had developed a few conidiophores with chains of six to ten spores.

Penicillium spiculisporum sp. nov.

Coloniis in agaro Solani tuberosi vel Phaseoli cultis, albis, rare instabiliter sulphureis, floccosis, extendentibus incerte; reverso albo aut cremeo. Conidiophoris sparis, ex hyphis aereis orientibus, 10–50 $\mu \times$ 2–2.5 μ, usitate unum verticillum 1–6 basidiorum vel rare 1–2 metulorum vel basidiorum et metulorum gerentibus; basidiis 11–16 $\mu \times$ 1.8–2.5 μ, contractis ad apices; conidiis ovatis, ellipticis vel globosis, hyalinis vel pallide glaucis in massa, 2.5–4 $\mu \times$ 1.8–2.5 μ, levibus. Peritheciis abundantibus, globosis, primo albis, diende cremeis vel sulphureis, citrinis, flavis vel luteis, 0.4–2 mm., nondehiscentibus, peridiis non parenchymaticis, ex tribus ordinibus hyphorum, compositis; ascis globosis, ellipticis vel pyriformis, 7.2–10.8 $\mu \times$ 6.3–7.7 μ, hyalinis, 6–8 sporis; ascosporis ovatis, ellipticis, hyalinis, spinulosis, 2.5–4 $\mu \times$ 1.8–2.8 μ. Coloniis gelatinam nonliquifacientibus. Odore nullo. Aeris temperatione optima 33–35° C. Culturae ex Gossypio herbacearo Anson Co., N. C., U. S. A.

CULTURAL CHARACTERS

Potato agar, good growth, spreading colonies with low, white, floccose surface; glucose or saccharose added, vigorous growth, many perithecia formed; surface white or transiently yellow, reverse white to cream.

Potato plugs, vigorous growth soon forming a dense mat of white mycelium, and, later, numerous white, cream and yellow perithecia.

Bean agar, same as for potato agar but somewhat less vigorous.

Beef peptone agar, colonies remain small, surface velvety with conidial fructifications arising from immersed hyphae.

Steamed rice, good growth, numerous white, cream, pinkish or yellow perithecia within 30 days.

Fifteen per cent. gelatin, sparse growth, not liquidified in 30 days at 28° C.

Milk, very little, if any, growth.

Czapeck's solution agar (nitrogen omitted) to which the following substances were added:

Saccharose 3 per cent., poor growth, no perithecia.

Glucose, 3 per cent., poor growth, no perithecia.

Maltose, 3 per cent., good growth, many sulphur colored perithecia at 14 days, reverse faint cream.

Lactose, 3 per cent., very poor growth, no perithecia.

Galactose, 3 per cent., good growth of rapidly spreading colonies, few sulphur colored perithecia at 14 days.

Glycerin, 3 per cent., good growth, many cream-colored perithecia at 14 days, reverse white and cream.

Armour's peptone, 3 per cent., poor growth, no perithecia, reverse cream.

Armour's peptone, 3 per cent., and saccharose, 3 per cent., vigorous growth forming a dense mat of mycelium, few perithecia, surface floccose and gray with conidial fructifications, reverse dark cream.

Asparagin, poor growth, saccharose added, very good growth, many small perithecia.

Urea, no growth.

Potato starch, fairly good growth, many small perithecia, colonies surrounded by a wide circle of clear media from which the starch has been dissolved by an enzyme.

Butterfat, very poor growth.

Temperature relation: On bean- or potato-saccharose agar growth is slow at 20° C., good at 28, optimum at 33–35, good at 40.

Color: White is the predominating surface color; however, when conidia form in profusion, as infrequently accurs in small areas, or over the entire surface when certain substances are

added to the substratum, the surface appears gray with faint shades of green and brown. Other pigments are associated with the perithecia. These bodies are at first white, and may remain so indefinitely, but very often they become cream, pinkish or some shade of yellow. All these colors may be present in a single tube of steamed rice. The yellow varies in shade from sulphur through lemon-yellow to yellow and may deepen to golden-yellow in our cultures (13). Yellow is often transitory and erratic coming on comparatively few, if any, perithecia of a given rice culture, and usually fading to cream or white within a week or infrequently deepening to golden yellow. On potato plugs yellow is less transitory and more certain to develop. The reverse of petri dish cultures is often cream in color and pink is frequently seen in small mycelial areas in rice tubes.

AGRICULTURAL EXPERIMENTAL STATION,
WEST RALEIGH, N. C.

REFERENCES

1. **Saccardo, P. A.** Sylloge Fungorum.
2. **Lafar, Franz.** Handbuch der Technischen Mykologie, 4: 219–234. 1906
3. **Engler, A., and Prantl, K.** Die Natürlichen Pflanzenfamilien. Teil 1, Abteilung, 1: 304–306. 1897.
4. **Thom, Charles.** Cultural Studies of Species of *Penicillium*. U. S. D. A. Bur. Animal Indus. Bull. 118. 1910.
5. **Sopp, Johan-Olson.** Monographie der Pilzgruppe *Penicillium* mit Besonderer Berücksichtigung der in Norway Gefundenen Arten. Skrifter utgit Videnskapsselskapet i Kristiania f. 1912, I Mat.-Nat. Cl. 1. Band, Nr. 11, Kristiania 1912: 208 pages 23 Taf., 1 Textfig.
6. **Sartory, A.** Etude d'un *Penicillium* nouveau, *P. Gratioti* n. sp. Annal. Mycol., 11: 161–165. *pl. 9.* 1913.
7. **Sartory, A., and Bainier, G.** Etude d'un *Penicillium* nouveau, *P. Herquei* n. sp. Bul. Soc. Mycol. France, 28: 120–126. *pl. 7.* 1912.
8. ———. Etudè de deux *Penicillium* nouveaux producteurs de pigments. Bul. Soc. Mycol. France, 28: 270–280. *pl. 13.* 1912.
9. ———. Etude morphologique et biologique des deux *Penicillium* nouveaux (espéces thermophiles). Bul. Soc. Mycol. France, 29: 367–377. 1913.
10. ———. Etudes morphologique et biologique d'un *Penicillium* nouveaux, *P. Petchii* n. sp. Annal. Mycol., 11: 272–277. *pl. 14.* 1913.
11. **Thom, Charles.** The *Penicillium* Luteum-Purpurogenum Group. Mycologia, 7: 134–142. *fig. 1.* 1915.
12. **Thom, Charles, and Turreson, G. W.** *Penicillium avellaneum.* A New Ascus-Producing Species. Mycologia, 7: 284–287. *fig. 1–3.* 1915.
13. **Saccardo, P. A.** Chromotaxia. Tabellae Colorum, 1894.

EXPLANATION OF PLATE 19

All drawings were outlined and as many as possible of the details put in with the aid of the camera ludica. The reduced magnification can easily be calculated from the scale given with each group.

Figs. 1–3. Hyphae showing nature of contents, septation and manner of branching.

Fig. 4. Sketch of fructifications.

Figs. 5–15. Conidiophores showing various types of fructifications found in potato glucose agar plates.

Fig. 16. Conidia grown in starch agar plate. Drawing made from water mount.

Fig. 17. Conidia grown on potato glucose agar. Drawing made from material stained with alcoholic carbol fuchsin and mounted in balsam.

Fig. 18. Conidial germination in a 20-hour old potato glucose agar culture at 28° C.

Fig. 19. Beginning stages in perithecial development.

Fig. 20. Microtome section of a cluster of perithecia grown on steamed rice. The letter "*a*" locates the inner, "*b*" the middle and "*c*" the outer layers of the peridium.

Fig. 21. Microtome section of small perithecia grown on potato glucose agar in petri dish.

Fig. 22. The portion marked "*x*" of Fig. 21 much more highly magnified. Note the character of the peridium and the clusters of ascospores among the fertile hyphae.

Fig. 23. Swollen hyphae from the center of a young perithecium.

Figs. 24–29. Asci and hyphal attachments.

Figs. 30–34. Asci containing ascospores.

Fig. 35. Ascospores highly magnified. Note the spines on the walls. Drawing made from spores stained with alcoholic carbol fuchsin and mounted in balsam.

Fig. 36. Ascospores germinating in a 24-hour old culture of beef peptone agar kept at 28° C.

Fig. 37. Camera lucida sketch of 48-hour old colony on beef petone agar. This colony grew from an ascospore.